ECOLOGY AND THERMAL INACTIVATION OF MICROBES
IN AND ON INTERPLANETARY SPACE VEHICLE
COMPONENTS

Thirty-fifth Quarterly Report of Progress

Order No. W-13411

October 1, 1973 - December 31, 1973

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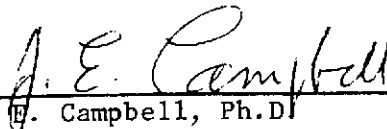Thirty-fifth Quarterly Report of Progress

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Introduction

Much of the effort of the personnel of the Cincinnati Food
Research Laboratories, Food and Drug Administration, assigned to this
project has been related to consultation, assistance, and collaborative
work with the Jet Propulsion Laboratory-Planetary Quarantine Laboratory
at Cape Kennedy, Florida.

Of particular interest in recent months has been the evaluation
of the proposed sterilization cycle for the Viking lander and the
influence of the moisture parameter on its effectiveness. One of the
major problems has been the evaluation of the Teflon strip data col-
lected in this work. This problem has been taken under advisement by
our Statiscian, and his conclusions are the subject of this Quarterly
Report.

A Note on the Statistical Assessment of a

Heating Cycle for Terminal Sterilization

An experimental sterilization facility has been developed at Space-craft Bioassay Laboratory, Cape Kennedy, Florida. This unit, described in the thirtieth Quarterly Report, simulates conditions that may be encountered during terminal sterilization of space vehicles. The purpose of this note is to discuss some of the statistical considerations in the design of terminal sterilization experiments.

At present spores are collected in the VAB from natural fallout on 1.5 square foot teflon strips. The initial population of about 200 spores per square foot is a mixture of organisms found in the same environment in which the space vehicles will be assembled. Final processing is expected to reduce the open surface concentration below 0.2 organisms per square foot. The equipment can be altered so that the heating cycles of different times, temperatures and moisture contents can be easily accommodated. Experiments must be designed to determine whether spore concentration has been adequately reduced by a given heating cycle. The following paragraphs discuss several aspects of the experimental situation.

Basic Design - Each experimental run consists of 33 teflon (1.5 sq. ft.) strips. Nine strips are negative controls and 24 strips are obtained from the VAB with about 300 spores per strip (200/sq.ft.). An initial count is obtained from eight other strips. After each heating cycle the strips are scored as positive or negative. The heating action on each strip is assumed to be independent. A negative result on one strip should not affect the result on a contiguous strip. The cultured strips are

incubated for 28 days. An MPN is estimated for runs where positive results are recorded. Equation (1) shows the computation of the MPN per square foot.

$$\text{MPN} = \frac{2.303}{1.5} \log_{10} \left(\frac{n}{s}\right) \tag{1}$$

n = number of strips

s = number of sterile strips

Table 1 shows the MPN per square foot for the experimental situation where n = 24.

Pooling of Runs - The initial counts are obtained on eight strips. If these counts are considered normally distributed under $\log_{10}$ transformation, then confidence limits can be computed. Rough limits can be computed to determine whether MPN estimates can be pooled. The variance of the $\log_{10}$ counts estimated from three runs (no. 2, 14, and 16 in Report #42 by CDC, Environmental Microbiology Section) is 0.03161. Thus the 99 percent confidence limit for a geometric mean of 200 (n = 8) would be 138 to 290.

Estimates of MPN for the three runs cited above are 0.029, 0.029, and 0.134. The variance of the $\log_{10}$ counts among runs is 0.14728. These results are from the same heating cycle and initial counts that do not differ significantly at the $\alpha = 0.01$ level.

Process Assessment - A series of runs can be observed to determine whether a given cycle yields results below 0.2 spores/square foot. Using the estimate of variance computed above, six runs would be needed to yield 99 percent confidence limits about the geometric mean. The limits would be about one half log cycle on either side of the mean. A series of runs with all negative results would be considered satisfactory since the lowest

MPN estimate is 0.029 per square foot or about one log cycle lower than the limit. A heating cycle with geometric mean and upper confidence limit below 0.2 spores/square foot would be acceptable.

Another series of experiments might consist of setting a cycle at a constant temperature and changing the humidity. A curve similar to the one drawn in Figure 1 could be generated. This experiment would indicate the upper limits of moisture that could be tolerated in a heating cycle. Other experiments can be designed in a similar manner.
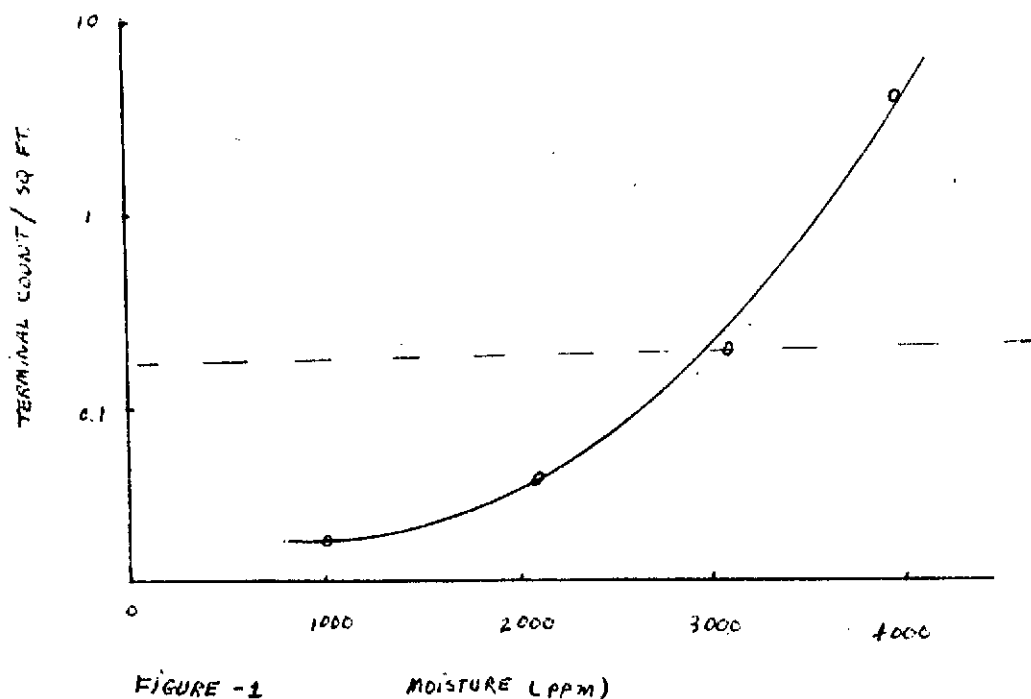

FIGURE -1   MOISTURE (ppm)

Table 1

Estimates of MPN Per Teflon Strip and Per Square Food for N = 24

Number of Sterile Units	MPN Per Strip	MPN Per Square Foot
23	0.043	0.028
22	0.087	0.058
21	0.134	0.089
20	0.182	0.122
19	0.234	0.156
18	0.288	0.192
17	0.345	0.230
16	0.406	0.270
15	0.470	0.313
14	0.539	0.359
13	0.613	0.409
12	0.693	0.462
11	0.780	0.520
10	0.876	0.584
9	0.981	0.654
8	1.099	0.733
7	1.232	0.822
6	1.387	0.924
5	1.569	1.046
4	1.792	1.195
3	2.080	1.387
2	2.485	1.656
1	3.179	2.119